**It takes a village to skew a lymph node**

Emily E Thornton[1] and Tal I Arnon[2]

[1] University of Oxford, MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine and Nuffield Department of Medicine, Oxford, UK
[2] University of Oxford, Kennedy Institute of Rheumatology, Oxford, UK;

Secondary lymphoid organs evolved to ensure that rare antigen-specific T and B cells receive activation signals in a timely and context-dependent manner. Lymph nodes (LNs) are the most common secondary lymphoid organs in our body. These structures function as a diffuse network of 'roadblocks' that traps pathogens and prevents their systemic dissemination (Qi et al., 2014). Because all LNs share a similar architecture, composed of distinct compartments organized by the same set of migratory cues, recirculating lymphocytes that enter these sites find them 'familiar' and easily navigate within them. Despite these commonalities, the anatomical location of each LN can greatly impact the type of response it induces. For example, T cells that are primed in the gut draining LN preferentially migrate to the lamina propria, indicating that activation in this location imprints gut-specific identity (Mora et al., 2003). Moreover, in some cases, depending on the precise tissue segment that the LN drains, its entire 'flavour' is skewed into a tolerogenic or inflammatory site (Muller et al., 2020). It is not fully understood how LNs acquire their unique anatomical identity. However, evidence shows that exposure to tissue-specific lymph born lipids and soluble factors, as well as encounters with specialized migratory DCs that sample the local environment, contribute to this process.

In this study Ataide, Knopper, and Cruz de Cases et al. asked whether other mechanisms contribute to establishing LN tissue identity. By studying individual LNs in isolation, they discovered that each contains a unique spectra of unconventional T cells (UTCs), populated from the tissue, which dictates variable cytokine landscapes and therefore influences the adaptive and innate immune responses that are triggered. They further showed that the UTC population, which primarily includes $\gamma\delta$T cells, natural killer T (NKT), and mucosal-associated invariant T (MAIT) cells, acts as a single functional unit that operates independently of antigen specificity or cell lineage to polarize LN cytokine profiles.

The authors started by exploring the UTC and cytokine compositions of lung, skin, and gut draining LNs. They found that the cytokines IL-17a and IL-4 were mostly derived from UTCs and were prevalent in the skin and lung draining LNs, but not in the gut (Figure 1, top). NKT cells, which were the main source of IL-4 in these sites, promoted immunoglobulin G1 (IgG1) germinal centre B cell differentiation specifically in the lung-draining LN, whilst having no effect in the gut-draining LN, supporting the idea that heterogeneity in UTC populations may contribute to shaping adaptive immune responses (Figure 1, bottom). To explore this possibility, the authors turned to single-cell RNA sequencing. They identified twelve clusters of UTCs that populated the lung, skin and gut-draining LNs, and they showed that these can be segregated into two main groups distinguished by CD62L (encoded by *Sell*, also known as L-selectin) expression, a hallmark of recirculating lymphocytes. Consistent with being recirculating, CD62L$^+$ clusters were represented similarly in the blood, while those that

lacked CD62L expression showed differential distribution across LNs, indicating limited recirculation potential. Critically, analysis of different tissues revealed that in each anatomical location the unique UTC signature that populated the tissue mirrored the UTC composition found in downstream draining LNs. These findings led the authors to propose that tissues dictate the specific transcriptional states of UTCs in local LNs.

How is this information relayed? One possibility that the authors considered, is that active migration of UTCs directly from tissues into their draining LNs contributes to this process. They therefore asked whether swapping the location of skin and gut draining LNs will result with a respective switch in their phenotypes. Indeed, when gut draining LNs, which lacked the non-migratory CD62L$^-$ UTC17 subset (ITG$\beta$7$^+$ CXCR6$^+$), were transplanted into the popliteal fossa, within 8 weeks a sizable population of CD62L$^-$ UTC17 appeared. Reciprocally, skin-draining LNs transplanted into the mesentery adopted the phenotype of a gut-draining LN, as indicated by loss of the CD62L$^-$ UTC17. Moreover, photoconversion of Dedra2-expressing cells in the skin or oral cavity showed that within 24 hours, 10% of the CD62L$^-$ UTC17 population in the skin or cervical LNs, respectively, were photoconverted, indicating that they recently migrated from these tissues. FTY720, which inhibits S1PR1 activity, blocked this effect, confirming previous studies showing a critical role for this receptor in UTC recruitment to LNs (Gray et al., 2013). Of note, the authors noticed that a specific UTC subset (LCK$^+$ $\alpha\beta$T CD62L$^-$) found in the inguinal LN, was absent from the skin but present in the adipocyte tissues surrounding it, highlighting the potential relevance of internal tissues for shaping UTC subsets in LNs.

Single cell RNAseq is often used to uncover differences between cell subsets and identify unique functions. However, when the authors used this approach, they discovered that similarly to UTCs in human tissues(Gutierrez-Arcelus et al., 2019), in the LN, the cells displayed a high degree of transcriptional homogeneity, which was maintained even after activation. UTC17 also occupied similar niches within the LN and migrated in a fairly homogeneous manner. This led the authors to consider the possibility that the entire population is functionally intertwined. To test this, they analysed mice deficient in $\gamma\delta$T cells (TCR$\delta^{-/-}$), NKT cells (CD1d$^{-/-}$), MAIT cells (MR1$^{-/-}$), or all three subsets together. Remarkably they found that in all cases, the total number of CD62L$^+$ and CD62L$^-$ UTC17 remained constant. The same results were obtained in draining LNs of TCR$\delta^{-/-}$, CD1d$^{-/-}$, or MR1$^{-/-}$ mice 4h after subcutaneous infection with *S. aureus*. Moreover, total levels of IFN$\gamma$ expression were also conserved in skin LNs and the spleen of mice subcutaneously or intravenously infected with *S. enterica*, respectively. Together, these findings suggest that LN UTCs act as one functional unit fully capable of compensating for the loss of one, two or even all three of its major subsets.

Finally, the authors asked whether skewing the cytokine milieu within individual LNs impacts the type of immunity induced. To address this, they infected mice with *S. aureus* via the subcutaneous or intraperitoneal routes. Remarkably, whilst both LNs were infected at similar levels, a robust recruitment of neutrophils could only be detected in the skin-draining LN, where UTC17 cells were present. Thus, unique UTC LN signatures correlate with a profoundly different type of immunity induced, even against the same pathogen.

The capacity of UTCs to establish residency in peripheral sites has been well documented (Fan and Rudensky, 2016). However, in recent years, studies from the Cyster lab show that some UTCs can recirculate; in the skin γδT Th17 cells continuously migrate to draining LNs, movement that is mediated by S1PR1 and which promotes their activation and subsequent homing to inflamed skin (Gray *et al.*, 2013; Ramirez-Valle et al., 2015). The study by the Kastenmüller lab not only extended the relevance of this homeostatic migration to a wider range of UTC lineages and tissues, but also uncovered a new important function it contributes to, imprinting tissue-identity within distinct LNs. Whilst in this study, the authors primarily focused on the implications of UTC trafficking to local LNs, it is likely that a similar mechanism contributes to a systemic mode of regulation by dictating UTC signatures in the spleen. Moreover, given the findings that some UTC subsets likely migrated from adipocyte tissues, which are known for their roles in sensing and adjusting immune responses to whole-body metabolic states (DiSpirito and Mathis, 2015), it may be interesting to ask whether these sites function as a reservoir for specialized UTCs poised to swiftly travel to downstream LNs in response to metabolic stress, providing an additional mechanism to shape LN polarity across the body.

Given their localization to interfollicular and subcapsular regions, UTC17s may come in direct contact with activated lymphocytes, providing an acute mode of regulation. Alternatively, they could be continuously interacting with local DCs or stromal cells, thus indirectly influencing downstream responses. Distinguishing between these options may be important, because the latter can lead to longer lasting changes, potentially contributing to the maintenance of chronic inflammatory diseases.

Although UTCs have been studied as individual subsets, evidence suggests that in tissues UTCs behave as a community, abandoning their unique individual identities imposed by lineage association and TCR specificity, whilst adopting a collective life-style (Constantinides and Belkaid, 2021). The study by the Kastenmuller group extends this notion to LNs, and further uncovers a new communal function for these cells; promoting an ongoing dialog between the tissue and its draining lymphoid organs. The findings that removal of all the 3 major UTC subsets (NKT, δγT and MAIT cells) had no effect on the overall phenotype is interesting and raises the question of which other UTCs or cell lineages are involved. More studies are needed to define the cellular and molecular mechanisms that regulate the stratification of UTCs in healthy, injured and aging tissues, and to explore if and how these changes imprint on the functionality of downstream LNs.

**Declaration of Interests**

The authors declare no competing interests.

**Figure legend**

Unconventional T cells (UTCs) migrate from tissues via the lymph in an S1PR1 mediated manner to draining lymph nodes. These UTC populations function collectively to confer unique tissue-determined cytokine profiles within the lymph nodes (top). Functionally, this affects immune outcomes, including IL-17 driving neutrophil recruitment in response to skin infection, and IL-4 driving geminal center IgG response in the lung-draining lymph node (bottom). Figure created with BioRender.com.

**References**

Constantinides, M.G., and Belkaid, Y. (2021). Early-life imprinting of unconventional T cells and tissue homeostasis. Science *374*, eabf0095. 10.1126/science.abf0095.

DiSpirito, J.R., and Mathis, D. (2015). Immunological contributions to adipose tissue homeostasis. Semin Immunol *27*, 315-321. 10.1016/j.smim.2015.10.005.

Fan, X., and Rudensky, A.Y. (2016). Hallmarks of Tissue-Resident Lymphocytes. Cell *164*, 1198-1211. 10.1016/j.cell.2016.02.048.

Gray, E.E., Ramirez-Valle, F., Xu, Y., Wu, S., Wu, Z., Karjalainen, K.E., and Cyster, J.G. (2013). Deficiency in IL-17-committed Vgamma4(+) gammadelta T cells in a spontaneous Sox13-mutant CD45.1(+) congenic mouse substrain provides protection from dermatitis. Nat Immunol *14*, 584-592. 10.1038/ni.2585.

Gutierrez-Arcelus, M., Teslovich, N., Mola, A.R., Polidoro, R.B., Nathan, A., Kim, H., Hannes, S., Slowikowski, K., Watts, G.F.M., Korsunsky, I., et al. (2019). Lymphocyte innateness defined by transcriptional states reflects a balance between proliferation and effector functions. Nat Commun *10*, 687. 10.1038/s41467-019-08604-4.

Mora, J.R., Bono, M.R., Manjunath, N., Weninger, W., Cavanagh, L.L., Rosemblatt, M., and Von Andrian, U.H. (2003). Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. Nature *424*, 88-93. 10.1038/nature01726.

Muller, P.A., Schneeberger, M., Matheis, F., Wang, P., Kerner, Z., Ilanges, A., Pellegrino, K., Del Marmol, J., Castro, T.B.R., Furuichi, M., et al. (2020). Microbiota modulate sympathetic neurons via a gut-brain circuit. Nature *583*, 441-446. 10.1038/s41586-020-2474-7.

Qi, H., Kastenmuller, W., and Germain, R.N. (2014). Spatiotemporal basis of innate and adaptive immunity in secondary lymphoid tissue. Annu Rev Cell Dev Biol *30*, 141-167. 10.1146/annurev-cellbio-100913-013254.

Ramirez-Valle, F., Gray, E.E., and Cyster, J.G. (2015). Inflammation induces dermal Vgamma4+ gammadeltaT17 memory-like cells that travel to distant skin and accelerate secondary IL-17-driven responses. Proc Natl Acad Sci U S A *112*, 8046-8051. 10.1073/pnas.1508990112.